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First report of *Eucoleus boehmi* in red foxes (*Vulpis vulpis*) in Denmark, based on coprological examination

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Abstract

Red foxes can be infected with diverse range of parasite species that can be transmitted to humans and pet animals, and the differential diagnosis is essential for veterinary and zoonotic risk assessment. In the present study, faecal flotation and microscopy of parasite eggs was done on 31 foxes originating from two distant localities in Denmark, the city of Copenhagen in the north east part of the island Zealand and from the southern part of the peninsular Jutland. In total, eggs of *Eucoleus boehmi* were recovered from a surprisingly high number of foxes (n = 22 samples; 71%). The findings represent the first observations of *E. boehmi* in Denmark, which is likely an overlooked infection of the upper respiratory tract of red foxes, which can be also found in other canids. Several morphological features of the eggs of *E. boehmi* can be used to distinguish it from other the closely related trichuroid eggs. Detecting cardiopulmonary parasites by faecal examination can be indicative of the presence of cardiopulmonary parasites based on the more sensitive post mortem analysis.

Keywords

Eucoleus boehmi, *Eucoleus aerophilus*, *Angiostrongylus vasorum*, *Crenosoma vulpis*, egg morphology, diagnosis

Introduction

Eucoleus (*Capillaria*) *boehmi* is commonly known as the nasal worm of dogs and foxes and was first recovered from a red fox in Germany (Supperer 1953). The life cycle of *E. boehmi* is not described yet and attempts to transmit *E. boehmi* to helminth-naive dogs by ingestion of *E. boehmi* eggs, ingestion of earthworms fed on eggs and intra-nasal inoculation of eggs were unsuccessful (Muchmore 1998). A cohort study for six and eight years on naturally infected dogs with *E. boehmi* revealed that the faecal output persisted despite a clean, isolated environment (Muchmore 1998). Infection with *E. boehmi* can cause mild respiratory signs such as sneezing and nasal discharge (Campbell and Little 1991), however, up to 88 worms were isolated from a naturally infected dog that did not exhibit any clinical signs (Muchmore 1998). The present paper describes the recovery of eggs of *E. boehmi* from red foxes in Denmark for the first time.

Materials and Methods

In the period from 2006 to 2008, 23 road-killed foxes were collected from Copenhagen greater area in the island Zealand, and eight hunted foxes from the southern region of the peninsular Jutland, that is geographically connected to mainland Europe. Foxes sampled were initially frozen at –80°C for at least 4 days for safety reasons. Trachea, heart and bronchi were opened with scissors and bronchioles and the rest of the lung tissue were dissected using a metallic needle to free nematodes. The dissected organs were washed with luke tap water (30–35°C) into a 212 µm sieve by means of a water jet, and collected worms were identified under a stereo microscope (x10) (Soulsby 1982). Unfortunately, nasal passages and frontal sinuses were not examined due to lacking of logistics related to skull examination (instruments and licence for sowing skulls). Faecal samples were collected from the colon of the animals sampled, and trichuroid eggs were isolated by flotation-sieving method modified after Davidson *et al.* (2009). Briefly two grams of faecal material were mixed with sugar-salt floatation fluid (specific gravity 1.2) and vortexed. The suspension was centrifuged at 1600 g for 10 minutes and the supernatant was poured through a 21 µm mesh size nylon filter. The retained material in the sieve was washed with distilled water and the remaining sediment was poured into a 3.5 ml petri-dish and examined using an inverted microscope at 40 × magnification. Once detected, trichuroid eggs were aspirated by glass pipettes

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and transferred to a glass slide and mounted with a cover slip and thereafter examined under light and phase contrast microscope (Olympus AX-70) at 200 × – 400 × magnifications. Differential diagnosis of trichuroid eggs was based on egg size, shape and color, plug shape, embryo and the surface structure of the outer egg-shell (Butterworth and Beverley-Button 1980; Campell 1991; Campell and Little 1991; Schoning *et al.* 1993).

Results and Discussion

By post mortem examination, three parasite species were detected: *Angiostrongylus vasorum*; *Eucoleus aerophilus;* and *Crenosoma vulpis* (Table I). Only one fox was free from cardiopulmonary parasites, 13 were infected with one species, 14 were infected with two species and three were infected with

Table I. Cardiopulmonary parasites detected in 31 foxes based on *post mortem* and faecal examination

		Results of post mortem examination			Results of faecal examination			
Area	**Animal code**	***Angiostrongylus vasorum***	***Eucoleus aerophilus***	***Crenosoma vulpis***	***Angiostrongylus vasorum***	***Eucoleus aerophilus***	***Eucoleus boehmi***	***Crenosoma vulpis***
Jutland	1		X			X	X	
Jutland	4		X	X			X	X
Jutland	7		X			X	X	
Jutland	9		X				X	
Jutland	11		X					
Jutland	18		X				X	
Jutland	48		X			X	X	
Jutland	49		X	X			X	
Zealand	6	X	X	X	X	X	X	
Zealand	17	X	X		X			
Zealand	25	X	X	X	X	X	X	
Zealand	78	X	X		X		X	
Zealand	79	X	X			X	X	
Zealand	81	X	X					
Zealand	82	X	X	X		X		X
Zealand	83						X	
Zealand	85	X	X			X	X	
Zealand	86		X			X	X	
Zealand	92	X			X		X	
Zealand	93	X			X		X	
Zealand	94	X			X		X	
Zealand	96		X			X		
Zealand	97	X			X		X	
Zealand	98	X	X					
Zealand	99	X	X			X		
Zealand	101	X	X		X	X	X	
Zealand	102	X	X					
Zealand	103	X	X		X	X	X	
Zealand	104		X	X		X		X
Zealand	105		X			X	X	
Zealand	107	X	X		X	X	X	
Total	**31**	**18**	**26**	**6**	**11**	**16**	**22**	**3**
Prevalence (%)	**100**	**58**	**84**	**19**	**35**	**52**	**71**	**10**

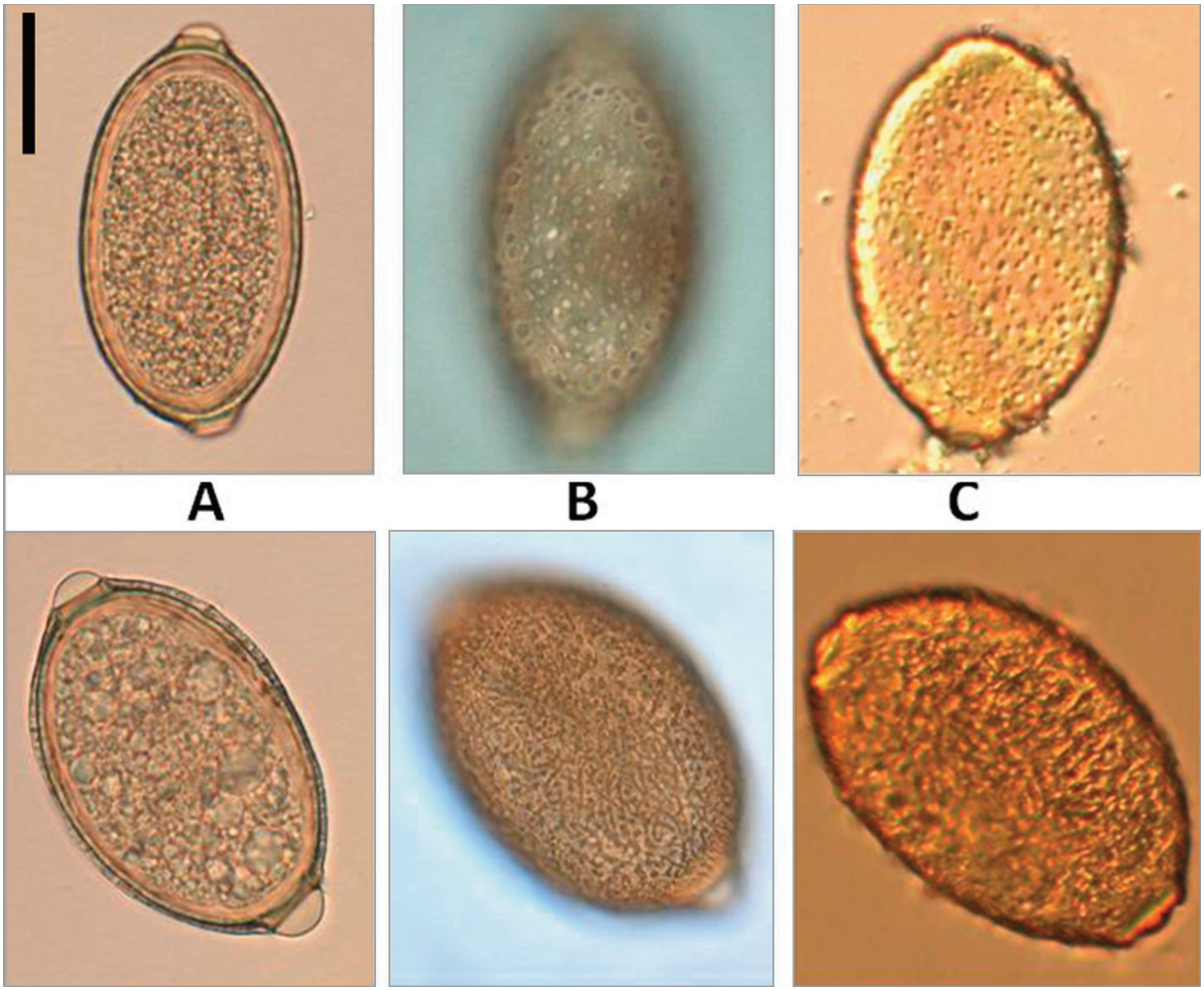

Fig. 1. Egg shell morphology of *Eucoleus boehmi* and *Eucoleus aerophilus.* Outer shell morphology of eggs of *Eucoleus boehmi* (upper row) compared to *Eucoleus aerophilus* (lower row) as seen using direct light microscopy (A), by fine adjustment focusing on the outer shell plane (B) or by using a phase contrast microscopy (C). Eggs were scaled to fit the presented scale bar on the upper left side of the figure (= 20 μm)

three species. Based on faecal examination, 22 samples contained eggs of *E. boehmi* (71%; Fig. 1), five of which belonged to foxes that did not have *E. aerophilus* at post mortem examination. Eggs of *E. boehmi* were 55.4–67.5 μm × 30.3–35 μm, whereas eggs of eggs of *E. aerophilus* were 60.7–64.3 μm × 28.5–30.4 μm. All foxes examined in this study were infected with at least one *Eucoleus* spp.

We noticed a marked difference in the prevalence of cardiopulmonary parasites based on faecal examination compared to that based on post mortem examination. Nonetheless, the recovery of lungworm larvae and/or eggs by faecal examination underestimated the post mortem recovery of *E. aerophilus* worms by 32%, *A. vasorum* by 23% and *C. vulpis* by 9% (Fig. 2). Over-representation of parasite eggs in faeces as a result of coprophagia could not be determined in the current study, but is not likely to affect the current results since none of the foxes that were free from a certain helminth species had parasite eggs or larvae of that species in its faeces, and also due to the underestimation of cardiopulmonary infections based on faecal examination. This underestimated detection of *A. vasorum* and *C. vulpis* might be caused by prepatent infections or the intermittent and irregular pattern of larval excretion (Willesen *et al.* 2004), or the possible damage of larvae when isolated by floatation medium (Traversa *et al.* 2010). Reduced recovery of nematode eggs could be a result of damaged eggs because of freezing and thawing or the use of improper floatation medium or techniques (Dryden *et al.* 2005).

The gold standard method for the recovery of viable lungworm larvae is the Baermann technique (Willesen *et al.* 2004). In this study, fox carcasses were already frozen and therefore implementing the Baermann technique was not valid. Instead, faecal flotation was found to be effective in isolating first stage larvae of *A. vasorum* (Al-Sabi *et al.* 2010; Schnyder *et al.*

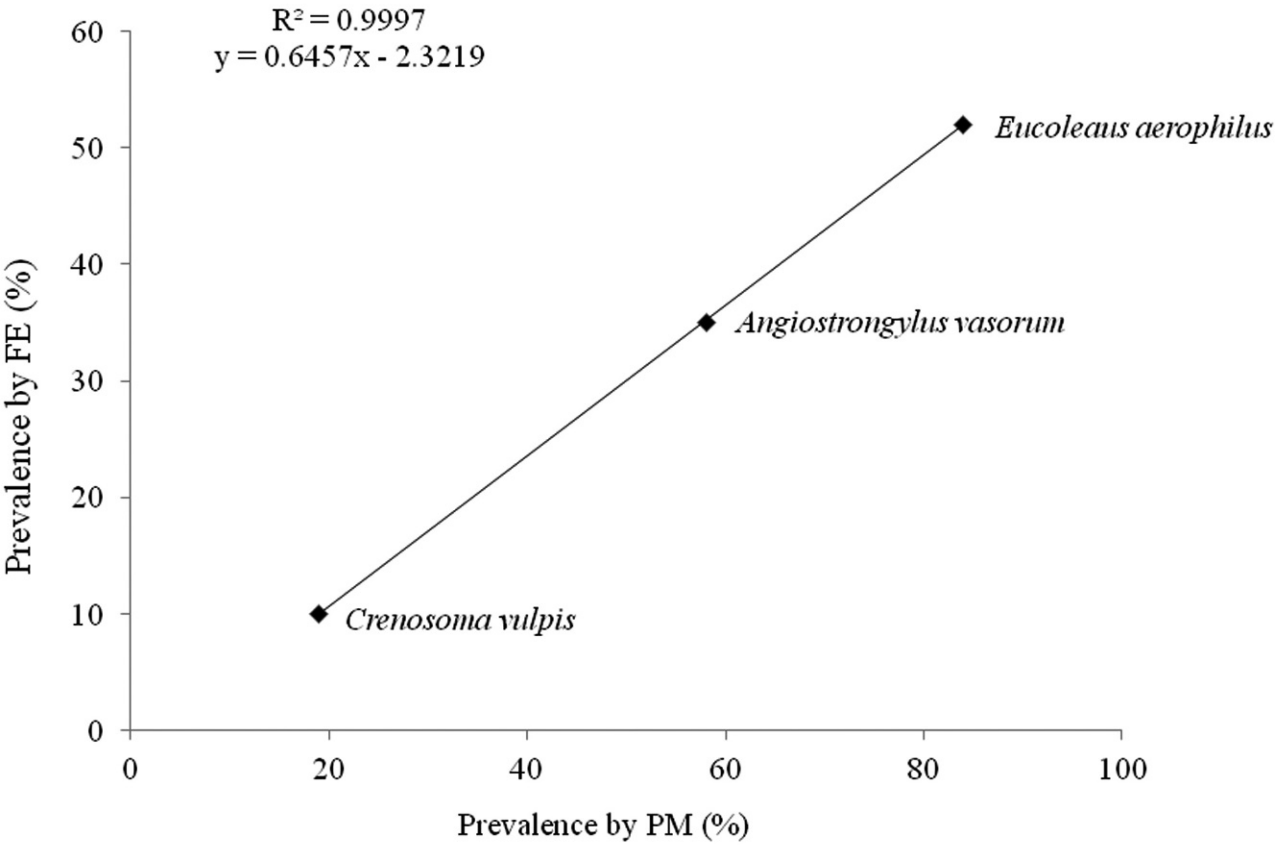

Fig. 2. Correlation between the prevalence of cardiopulmonary parasites based on post mortem and faecal examination methods. Correlation coefficient (R^2) presented on the figure indicates strong positive correlation between the reported prevalence of cardiopulmonary parasites of 31 foxes based on post mortem analysis (PM) and that reported by faecal egg isolation (FE) method. Correlation equation can be used to predict the prevalence based on faecal examination

2011), although it is not the preferred technique (Conboy 2009). Despite that, the strong linear association ($R^2 = 1$) between the two methods suggest that faecal examination can be used for estimating the presence of cardiopulmonary parasites in foxes based on environmental sampling of faeces, and thereby reducing the costs and logistic requirements for performing post mortem analysis of carcasses. However, we could not predict the presence of *E. boehmi* worms in foxes in Denmark due to lacking comparative data on recovery of worms by post mortem examination.

The reported size range of trichuroid eggs in the literature was markedly overlapping (Fig. 3), therefore other morphological features should be used to differentiate between these eggs. Eggs of *Trichuris vulpis* are characterized by its relative larger size, brownish to yellowish color, its polar plugs are symmetrically positioned in relation to the axis of the egg, the surface of its eggs is smooth and the presence of characteristic rings on the basis of the plugs (Campbell 1991; Campell and Little 1991; Schoning *et al.* 1993; Conboy 2009; Traversa *et al.* 2011; Di Cesare *et al.* 2012). In this study, the colon and the urinary bladder of the foxes sampled were not examined and eggs of *T. vulpis* and *Personema plica* were not detected. Based on the reported low prevalence of *T. vulpis* in foxes in Denmark; 0.5% of 1040 foxes (Saeed *et al.* 2006), and because the sample examined in this study was limited, the probability of detecting *T. vulpis* was extremely low. On the other hand, *P. plica* is very abundant in foxes in Denmark; 81% of 1040 foxes (Saeed *et al.* 2006). Despite that *P. plica* is a bladder nematode, its eggs can be accidently ingested during grooming and therefore could be, infrequently, found in faeces of infected animals (Campbell 1991). However, faecal examination is not the typical methodology for detecting infections with *P. plica*, but if present, the eggs can be clearly distinguished morphologically from other trichuroid eggs (Campbell 1991).

The ability to differentiate between *E. boehmi* and *E. aerophilus* is important because they differ in terms of pathogenicity and zoonotic potential (Traversa *et al.* 2010; Di Cesare *et al.* 2012). Furthermore, mixed infections with these two species naturally occur (Di Cesare *et al.* 2012); based on faecal analysis in this study, 12 out of 31 foxes (39%) had mixed infections with *E. boehmi* and *E. aerophilus*, and another study based on post mortem examination reported mixed infections in 94% of 88 foxes (Davidson *et al.* 2006). The bipolar plugs in eggs of *E. boehmi* were previously said to be blister-like and more protruding outwards than those of *E. aerophilus* (Campbell 1991; Campell and Little 1991; Con-

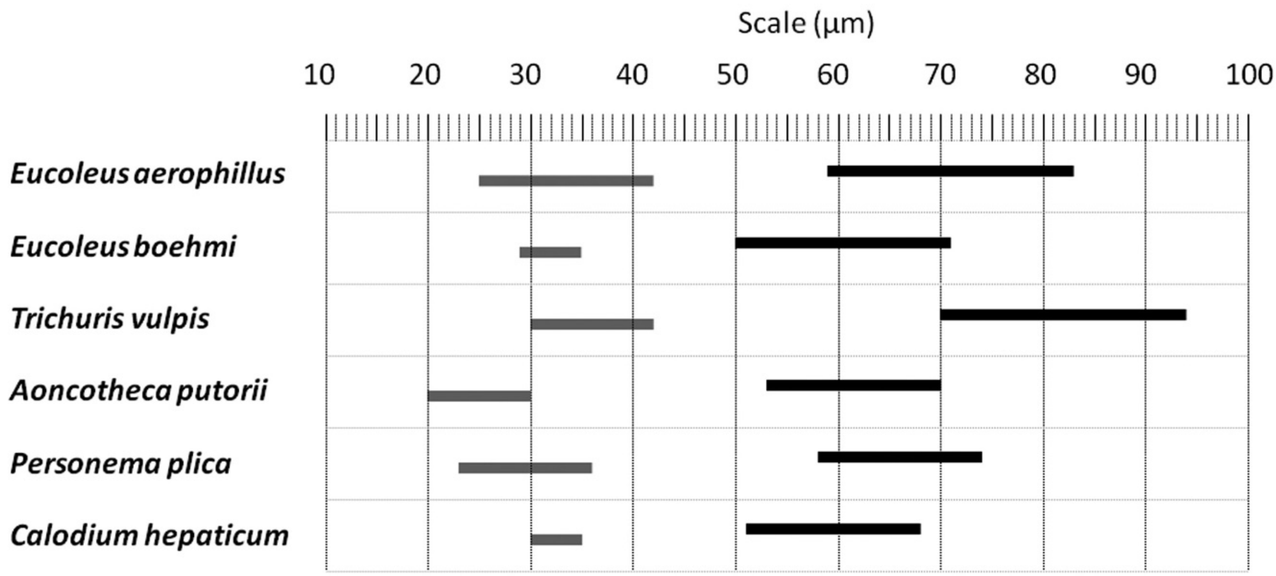

Fig. 3. Reported size ranges of trichuroid eggs in the literature (Supperer 1953; Butterworth and Beverley-Burton 1980; Campbell 1991; Campell and Little 1991; Schoning *et al.* 1993; Moravec 2000; Romashov *et al.* 2000; Conboy 2009; Traversa *et al.* 2010; Di Cesare *et al.* 2012). Size range of length (black) and width (grey) of trichuroid eggs commonly recovered in many carnivore species.

boy 2009), but we did not see this feature constantly and it was hard to evaluate (Fig. 1). The embryo is said to be more differentiated in *E. boehmi* leaving more space inside the egg compared to eggs of *E. aerophilus* (Campell and Little 1991). However, Muchmore (1998) reported that 90% of *E. boehmi* eggs freshly isolated from faeces had multicellular embryo with space between it and the egg shell, and 6% of the eggs had undifferentiated zygotes that completely filled the interior space of the eggs, while the rest were eggs that had vermiform to fully developed larvae. The most important characteristic of *E. boehmi* eggs is the pitted appearance of the shell that is given by the presence of several pits that are irregularly scattered (Fig. 1). The pits on *E. boehmi* eggs are of different sizes and are generally round to oval, which is different from the netted appearance of eggs of *E. aerophilus* (Supperer 1953; Butterworth and Beverley-Burton 1980; Campbell 1991; Campell and Little 1991; Schoning *et al.* 1993; Romashov *et al.* 2000; Conboy 2009; Traversa *et al.* 2010; Magi *et al.* 2012).

Detection of *E. boehmi* in this study was done by thorough microscopic examination of the eggs which allowed for separation of *E. boehmi* from other trichuroid species. Only a few studies considered the morphological difference when faecal eggs were examined (Campbell and Little 1991; Traversa *et al.* 2009). Hence, the presence of Capillarid eggs was often referred to as *Eucoleus* (*Capillaria*) spp., while other studies stated that *E. boehmi* might have been confused with other trichuroid eggs (Campbell and Little 1991; Lassing *et al.* 1998; Sreter *et al.* 2003; Conboy 2009; Traversa *et al.* 2010). A few studies based on post mortem analysis of cardiopulmonary organs in foxes included steps of examining the nasal and cranial sinuses (Table II), leaving a gap in our knowledge on *E. boehmi* prevalence that might be under estimated (Campbell and Little 1991; Manas *et al.* 2005). Periodical egg

Table II. Reported prevalence of fox cardiopulmonary parasites in Europe by post mortem or faecal examination (*)

Reference	Country	Sample size	Nasal and frontal sinuses examined	*Eucoleus boehmi*	*Eucoleus aerophilus*	*Crenosoma vulpis*	*Angiostrongylus vasorum*
Saeed *et al.* 2006	Denmark	1040	No	Not reported	74.1	17	48.6
This study	Denmark	31	No	71*	84	19	58
Rajkovic-Janje *et al.* 2002	Croatia	85	No	Not reported	4.7	28.2	1
Gortazar *et al.* 1998	Spain	81	No	Not reported	34.8	3	20.7
Borgsteede 1984	Netherlands	139	No	Not reported	46.8	5	0
Eira *et al.* 2006	Portugal	62	No	Not reported	4.8	3.2	16.1
Magi *et al.* 2009	Italy	129	No	Not reported	7	14.7	7
Sreter *et al.* 2003	Hungary	100	Yes	8	66	24	5
Davidson *et al.* 2006	Norway	181	Yes	51 (n = 174)	88 (n = 181)	58 (n = 181)	0

excretion of *E. boehmi*, peaking every five to seven weeks (Schoning *et al.* 1993), also complicates its detection by faecal examination. Therefore, faecal samples collected on consecutive days from alive animals should be examined; otherwise collecting worms and eggs of *E. boehmi* could be done by applying nasal washing (Schoning *et al.* 1993), but not nasal swabs only (Magi *et al.* 2012). A study on 51 foxes in Canada recovered eggs of *E. aerophilus* in 68.6% of the examined faeces, whereas worms were recovered by post mortem in only 49% of the foxes (Nevarez *et al.* 2005). It was therefore concluded that prevalence of *E. aerophilus* could have been overestimated since only histological examination of lungs was practiced without examining nasal and frontal sinuses.

This is the first report of *E. boehmi* in foxes from geographically distant locations in Denmark, suggesting that the parasite may be relatively prevalent and widespread. Infections with *E. boehmi* could be found in companion dogs but with low prevalence (Conboy 2009; Traversa *et al.* 2010; Di Cesari *et al.* 2012; Magi *et al.* 2012). The implications of the present high prevalence of *E. boehmi* in foxes could not be assessed in this study, however transmission of *A. vasorum* between foxes and dogs in endemic areas was demonstrated by molecular analysis (Jefferies *et al.* 2010). Another factor that could perpetuate *E. boehmi* in nature is its probable transmission between wider range of wild and domesticated carnivore species. Together with the given limitation of recovering eggs of *E. boehmi* from faeces, the presence of this parasite in companion carnivores might be more frequent than expected (Conboy 2009).

Conclusions

Infections with *Eucoleus boehmi* are prevalent in foxes in Denmark and could be more abundant among other carnivores, but overlooked by microscopy due to the overlapping morphological similarities between trichuroid eggs. Faecal examination of wild and domesticated carnivores should include steps of examining the egg-shell for differentiating trichuroid infections.

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